A 65 bp duplication/insertion in exon II of the β globin gene causing β^0-thalassemia

We describe a patient originating from Ghana who had combined heterozygous -$\alpha^{4.2}$ thalassemia, $\alpha\alpha\alpha^{anti3.7}$ triplication, the common δ globin variant HbA_2' and a new 65 bp duplication/insertion in exon II of the β globin gene causing β^0-thalassemia.

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We report a new β-thalassemia mutation that was detected in a 30-year old man originating from Ghana. Informed consent to all investigations was obtained. His hematologic parameters were as follows: hemoglobin 133 g/L, packed cell volume 0.42 L/L, red blood cell count 6.74×10^{12}/L, mean cell volume 62.3 fL, mean cell hemoglobin 19.7 pg, reticulocytes 6.3% or 425×10^9/L. Total bilirubin concentration was 27.3 µmol/L. The blood smear showed weak basophilic stippling but no Heinz bodies. Occasional target cells, ovalocytes and a few tear drops could be identified. Cation exchange high performance liquid chromatography[1] showed 2.2% of HbA_2 as well as 1.5% HbA_2' giving a total HbA_2 concentration of 3.7%. The HbF level was 1.2% and ferritin was slighly elevated (543 µg/L). Screening for α-thalassemia, conducted according to Liu *et al.*,[2] showed the heterozygous -$\alpha^{4.2}$ deletion as well as the $\alpha\alpha\alpha^{anti\ 3.7}$ triplication. The nature of the δ variant (δcd16 GGC→CGC) was confirmed by direct sequencing as described elsewhere.[3] The elevated HbA_2 concentration together with the hematologic indices prompted us to search for an additional β-thalassemia mutation. DNA extraction and polymerase chain reaction (PCR) analysis were done using standard methods, sequencing with Big Dye Version 1.1 chemistry on an ABI 310 automated sequencer (Applied BioSystems, Rotkreuz, Switzerland). Amplification of the whole β globin gene from nt - 109 to + 1949 3' UTR (all nt positions are given relative to the CAP site=nt 1) using primers Common C and B designed by Old *et al.*[4] produced a 2107 bp long amplicon. The corresponding PCR product on agarose gel showed an unresolved double band. An insertion/deletion type overlay was detected in exon II by direct sequencing with primer IIIAL (nt 171 to 191). We, therefore, amplified a shorter PCR product of 472 bp with primers IIIAL and HF 8 (nt 642 to 623). The elongated fragment was cut out from acrylamide gel, put directly in a semi-nested re-amplification reaction with internal primers IIIAL and IIIAR (nt 525 to 506) and sequenced using the same primers. A duplication of a 65 bp long piece of exon II from nt 367 to nt 431 was demonstrated. The sequence data of this insertion are shown in Figure 1. The duplication/insertion leads to an altered amino acid sequence starting at codon 84 (T→L). After leucine, an additional 25 amino acids, MARKCSV-PLVMAWLTWTTSRAPLPH, followed by a stop codon at nt 511 to 513, are coded for by the mutant sequence. This leads to an altered β chain of only 106 residues. We were not able to detect any other mutant hemoglobin or chains than the heterozygous HbA_2' by ion exchange or reversed phase chromatography. This duplication/insertion obviously leads to a β^0 thalassemia phenotype.

To date only three duplication/inserts of more than 20 nucleotides have been described for the globin genes. Two of them, a 86 bp and a 45 bp duplication, were

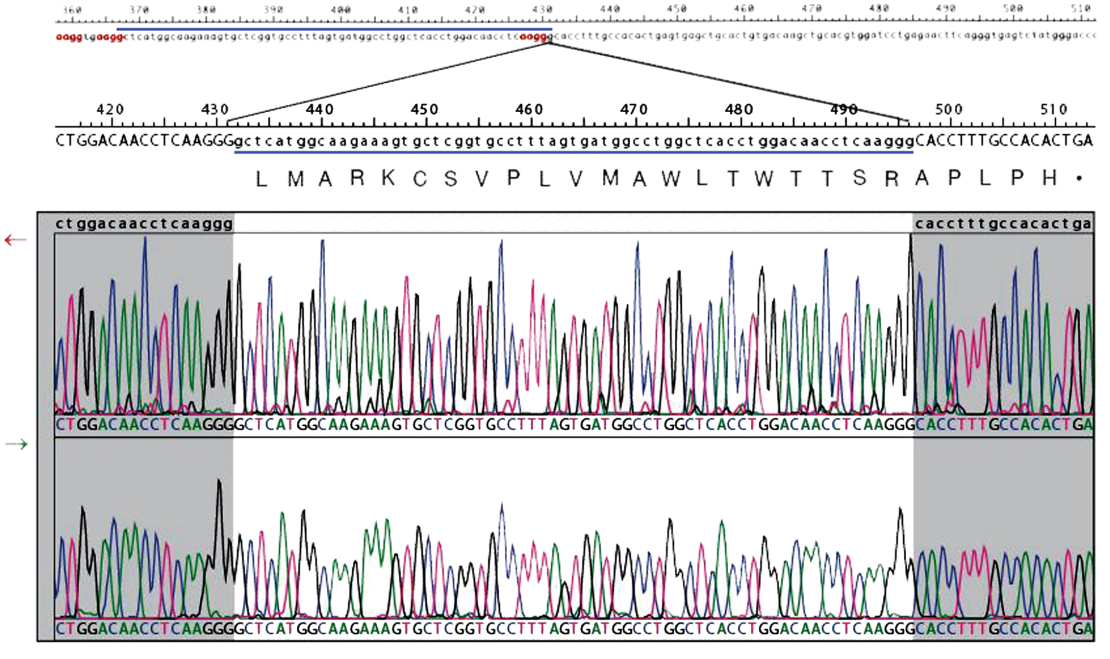

Figure 1. Sequencing data of the 65 bp insert. The wild-type β globin sequence is shown at the top: blue bars represent the sequence which is duplicated, the homologous flanking nucleotides are marked in red. The shaded area represents the wild-type part of exon II, the clear area shows the 65 bp duplication. Nt 432 (G) is the first base of the insert, it is thus the duplication of nucleotide 367 of the wild-type β globin. Forward and reverse sequences are shown. The mutant amino acids starting with L at position 433-435 are shown above the sequence.

found in a Portuguese and a Maori family respectively, and both led to β^0-thalassemia.[5,6] Both inserts code for truncated β chains that cannot been found in circulation and thus give rise to a β^0-thalassemia phenotype. Another similar mutant in the α1 gene, a 21 bp duplication/insertion[7] give rises to an α^+ thalassemic phenotype by coding for a highly unstable α1 globin variant. Possible mechanisms have been postulated for these duplications events. In the case of our patient in both flanking regions of the insert there are 4 nt repeat homologies AAGG – two on the 5' side and one on the 3' side - as indicated in Figure 1. The most plausible mechanism leading to the mutation described here is a backward replication slippage mechanism as described by Chen *et al*.[8] The same should be the case for the Portuguese insertion[5] and the one described by Waye *et al*.,[7] whereas the Maori mutation[6] might be caused by a different mechanism given that it lacks homologies in the flanking regions.

The present mutation produces the hematologic picture of a β^0- thalassemia carrier. The relatively high reticulocyte count and the patient's minimal splenomegaly are the only indications that this duplication could even lead towards a more severe phenotype.[8] The patient has been living in Switzerland since 2003. His parents, five siblings and three children live in Ghana. We have unfortunately not been able to obtain any blood data from them. Analyzing other family members will be necessary to define the clinical impact of the present duplication/insertion more precisely.

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